

Annealing Temperature Project N. _____
pM29 / dif. enzymes / dif. primers / dif. Book No. _____

11/28/94

Page N. _____
 Purpose: To check all different annealing temps to get rid of 97% mis priming during pM29 amplification

Temps checked 58°, 60°, 62°, 65°

56° gave more non specific bands than 58°. pg 102-

checked all three primer set } Soil - II new dv
 " " old dv 2728 + 29

Amplified with Tag, Tag + DV, DV alone.

200 μM dNTP

1 μM primer

200 μg template

2 μl ^{10X} buffer (from buffer) - used Deep Vent buffer

prepared cocktail for 80 Rx

added primers separately, adding respective enzymes.

lane *	1 - 8	Tag	old dv	each combination in duplicate
9 - 16			New dv	annealed at different
17 - 24			" - dv	temp 58, 60, 62, 65°
25 - 32	1 : 0.01 DV	old dv		
33 - 40		new dv		94° 3'
41 - 48		" - dv		30 (94°, 30°, X 30°, 72°, 3°)
49 - 56	Deep Vent	old dv		
57 - 64		new dv		72° 10'
65 - 72		" - dv		4° 20 min
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To Page No. _____

Signed & Understood by me,

Date 12/19/94

Invented by

Date

Recorded by

11/28/94

L. Subramanian

Project No. _____

Book No. _____

TITLE _____

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NO. 1

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Result: The only thing that worked:

Tag

Tag } old dV primaries at 58°
Tag + DV }

Tag + 0 v

Deep Vent / non dV / at all annoying Temp means?

all samples discarded

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Witnessed & Understood by me,

Date

Date

Invent d by

Recorded by

rk Jikme sunam

Date

11/29/94